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13. ABSTRACT *(Maximum 200 Words)*

This project aims to determine the roles of bioenergetic dysfunction and oxidative stress in mechanisms of neurodegeneration in Huntington's disease (HD) and familial amyotrophic lateral sclerosis. Studies in this second year extended the first year's investigations into cerebral glucose metabolism in mutant mouse models of HD. We found increases in cerebral glucose use in forebrain regions of 4 month-old *Hdh* "knock-in" mice expressing a mutant 92 glutamine stretch in huntingtin protein (Hdh^{Q92}), relative to levels in wild-type mice (Hdh^{Q7}). Metabolic changes precede pathologic changes and show a gene-dosage effect, homozygote Hdh^{Q92}mice showing larger magnitude changes than heterozygotes. We attempted to measure similar parameters in R6/2 HD mice, but experiments were hindered by the diabetic profile of these mice. However, findings in another HD mouse model (N171-82Q) also show pre-symptomatic cerebral glucose use elevations associated with the gene mutation. Results from years 1 and 2 suggest that mutant huntingtin expression induces metabolic abnormalities that precede symptom onset and pathological events in these mouse HD models. Further, the occurrence of metabolic changes is dependent on polyglutamine repeat length and gene dosage. These findings represent significant progress towards the original goals of this proposal.

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Susan Brown 7.31.01

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5. INTRODUCTION

The overall goal of these studies is to gain insight into the roles of energy metabolism and oxidative stress in the etiology of neuronal degeneration in Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). HD experiments utilize two mouse models of HD, *Hdh* "knock-in" mice and R6/2 transgenic mice, which express abnormally expanded polyglutamine (Q) stretches in huntingtin protein (encoded by expanded CAG repeats). ALS experiments utilize one transgenic mouse model of familial ALS, G93A mice over-expressing human mutant Cu/Zn superoxide dismutase (SOD1). We aim to determine the chronological order and relative contributions of bioenergetic defects and oxidative damage in the cell death mechanisms in these models. Studies in the first year employed [^{14}C]-2-deoxyglucose (2-DG) *in vivo* autoradiographic measurement of local cerebral metabolic rates for glucose ($lCMR_{glc}$) in conscious freely moving mice, and spectrophotometric assays of metabolic enzyme activities in post-mortem brain . We found reduced cerebral glucose use in several forebrain regions of the G93A FALS mice by 60 days of age – a time point preceding the onset of the first pathological changes in these animals (mitochondrial morphological changes at 70-80days). In addition, complex I activity is increased in G93A forebrain at the same time point, consistent with the defect seen in FALS A4V patients with a SOD1 mutation. In the *Hdh* "knock-in" mouse model of HD we found that cerebral glucose utilization was not altered from wild type levels by expression of 50 polyglutamines (Hdh^{Q50} mice), measured at 4 months of age (a timepoint chosen to precede pathologic changes, notably huntingtin aggregate formation, in this mouse line). However, alterations in the activities of the mitochondrial electron transport chain enzymes complexes I, II-III and IV were evident in cerebellum from Hdh^{Q50} and Hdh^{Q92} mice at this time point. In the second year of this project we extended these studies to assess parameters of cerebral energy metabolism in HD mouse lines expressing longer polyglutamine repeat stretches in mutant huntingtin protein; namely, Hdh^{Q92} mice (92 glutamines) and R6/2 mice (~145 glutamines). The specific experiments to be performed in Year Two were:

1) Measurement of the effects of increasing CAG repeat number on $lCMR_{glc}$ in *Hdh* knock-in mice (90 versus 48 CAG repeat length).
2) Measurement of $lCMR_{glc}$ in the R6/2 mouse model of HD. Analysis of the temporal progression of any glucose use changes by measurement at 42d, 56d and 84d of age.
3) Measurement of electron transport chain enzyme activities in R6/2 HD mice.
4) Nuclear magnetic resonance (NMR) *in vivo* spectroscopic imaging of lactate levels in *Hdh* and R6/2 mouse brains.

NB: As described in Section 6, in the course of this project we ascertained that R6/2 HD mice develop diabetes, preceding HD symptom onset. The nature of the associated alterations in glucose handling make this a poor model for measurement of cerebral glucose use. Therefore we were unable to perform 2-DG studies in these mice. However, we undertook 2-DG measurements in another HD mouse model over-expressing a human huntingtin fragment, the N171-82Q line. Results in this model are briefly described in Section 6.

6. BODY

The pathogenetic mechanism in Huntington's disease (HD) is still unclear, however *in vivo* and *in vitro* studies implicate the involvement of bioenergetic defects in the disease process. Attempts to ascertain the role of energetic dysfunction in pathogenesis have been greatly enhanced by the development of several different transgenic mouse strains expressing the huntingtin gene mutation underlying HD, which allow determination of metabolic parameters over the life-span of these animals. Strains differ in terms of the site of mutant gene incorporation, CAG repeat length, copy number, and promotor used, and these differences are reflected in the phenotypes of the mice generated. Studies in this project utilize *Hdh* CAG knock-in mice (White et al., 1997) and R6/2 transgenic mice overexpressing an N-terminal fragment of human mutant huntingtin, including a 145 polyglutamine repeat stretch.

Objective #1: **To investigate the effects of increasing CAG repeat number on local rates of cerebral glucose use ($lCMR_{glc}$) in a mouse model of HD expressing expanded CAG repaets in murine *HD*: *Hdh* knock-in mice (7, 48 and 90 CAG repeats).**

Cerebral Glucose Use in the Hdh "Knock-in" Mouse Model of HD: Hdh CAG knock-in mice (White et al., 1997) were developed by inserting CAG repeats into exon 1 of the murine huntingtin homologue (*Hdh*) to generate a set of precise genetic HD mouse models which accurately express mutant huntingtin protein. In this study we used [^{14}C]-2-deoxyglucose in vivo autoradiography in four month-old Hdh knock-in mice to determine:

- if energy metabolism is altered by expression of expanded polyglutamines in huntingtin protein,
- the effect of increased CAG repeat number (48 and 90); and
- the effect of gene dosage on glucose use, at a time point preceding the onset of any behavioral changes and neuronal intranuclear inclusion (NII) formation in these mice.

We used a modification of the [^{14}C]-2-deoxyglucose *in vivo* autoradiography technique developed by Sokoloff and colleagues (1977; Browne et al., 1999b) to measure local rates of cerebral glucose use ($lCMR_{glc}$) in *Hdh* "knock-in" mice. The [^{14}C]-2-deoxyglucose procedure facilitates localization and quantitation of $lCMR_{glc}$ in discrete anatomical regions throughout the CNS of conscious animals. It's utility is based on the premise that glucose is the primary energy source for cerebral cells, but brain tissue has a minimal capacity to store carbohydrates and therefore relies on glucose extraction from the circulation to fulfill energy demands. Hence, regional measurement of the rate of uptake of a radiolabelled glucose analog, ^{14}C-2-deoxy-glucose, from the blood allows *in vivo* estimation of local rates of energy metabolism. Changes in glucose use generally reflect alterations in cerebral functional activity.

Cerebral metabolic rates for glucose in *Hdh* mice expressing 50 glutamines, both homozygous and heterozygous for the transgene (Hdh^{Q50}, 48/48 and 48/7 CAG repeats, respectively) were reported in the

Year One progress report. Here we report the results of 2-DG studies in *Hdh* mice expressing 92 glutamines (Hdh^{Q92}, 90/90 and 90/7 CAG repeats) and in normal wild-type littermates (Hdh^{Q7}, 7/7 CAGs).

Physiological Variables: Arterial plasma glucose concentration, *p*O2, *p*CO2 and *p*H were measured 35 minutes into the procedure (10 minutes before animal decapitation) to determine the physiological status of the animals. There were no significant differences in the levels of any of these measured parameters between 7/7 wild type mice and levels in 90/7 and 90/90 mice ($p > 0.05$, ANOVA, followed by Fisher's PLSD). All measured parameters were within accepted normal ranges, indicating that there are no physiological effects associated with the *Hdh* mutation that might alter the interpretation of glucose use results. In separate studies we assessed glucose tolerance in *Hdh* mutant mice, in light of reports of diabetes in the R6/2 HD mouse line . We found that neither *Hdh* Q7, Q50, Q92 or Q111 mice showed any evidence of hyperglycemia or abnormal glucose handling.

Glucose Utilization: Glucose use values in 21 brain regions examined are presented in Table 1. A comparison of glucose use values between Hdh^{Q92} and Hdh^{Q50} mice is shown in Figure 1.

Table 1: Local Cerebral Glucose Utilization ($lCMR_{glc}$) in Hdh^{Q92} CAG Knock-in Mice

REGION	7/7	90/7	90/90
Frontal Cortex	52.3 ± 6.6	60.1 ± 3.6	78.2 ± 6.7 ** †
Parietal Cortex	50.0 ± 7.0	58.5 ± 4.0	75.3 ± 7.6 *
Anterior Cingulate Cortex	74.2 ± 11.8	78.4 ± 7.3	99.2 ± 8.7
Auditory Cortex	51.0 ± 6.0	64.9 ± 5.4	81.5 ± 8.7 *
Striatum: Dorsolateral	57.8 ± 7.1	61.5 ± 5.9	81.1 ± 6.2 * †
Ventromedial	55.8 ± 7.3	58.1 ± 6.1	76.0 ± 6.7
Globus Pallidus	32.3 ± 4.1	38.3 ± 2.5	50.3 ± 7.8 *
Hippocampus: CA1	30.0 ± 4.1	41.3 ± 3.6	50.3 ± 6.9 *
CA3	41.2 ± 5.4	52.7 ± 3.4	66.3 ± 9.6 *
Dentate Gyrus: Molecular Layer	47.7 ± 6.1	55.8 ± 4.6	73.2 ± 6.0 ** †
Dorsolateral Geniculate Body	49.0 ± 6.6	56.9 ± 4.8	71.5 ± 10.1
Medial Geniculate Body	56.8 ± 7.2	67.2 ± 3.7	75.0 ± 9.2
Superior Colliculus: Superficial Layer	46.8 ± 6.1	54.9 ± 4.5	68.2 ± 6.8 *
Deep Layer	46.2 ± 6.1	55.1 ± 3.3	68.1 ± 6.3 *
Internal Capsule	12.4 ± 1.7	18.6 ± 1.2	24.2 ± 6.6 *
Thalamus: Dorsomedial	54.7 ± 8.0	65.6 ± 4.4	84.3 ± 10.2*
Ventromedial	39.6 ± 5.9	47.3 ± 4.0	58.3 ± 7.9 *
Substantia Nigra: *pars reticulata*	28.6 ± 4.0	37.4 ± 2.0	44.1 ± 6.5 *
pars compacta	48.5 ± 7.7	58.5 ± 4.3	69.7 ± 11.2
Cerebellum: Grey matter	31.0 ± 4.2	37.0 ± 2.4	47.5 ± 7.5 *
White matter	21.2 ± 2.3	27.7 ± 1.8	29.8 ± 4.6

$lCMR_{glc}$ (nmol/100g/min) in homozygous (90/90) and heterozygous (90/7) Hdh^{Q92} knock-in mice; and wild-type (7/7; Hdh^{Q7}) littermate controls. Data presented as mean ± SEM, (n=4-6 per group).
* $P < 0.05$, ** $P < 0.01$ relative to levels in 7/7 mice; † $P < 0.05$ relative to 90/7 mice (ANOVA, followed by Fisher's PLSD).

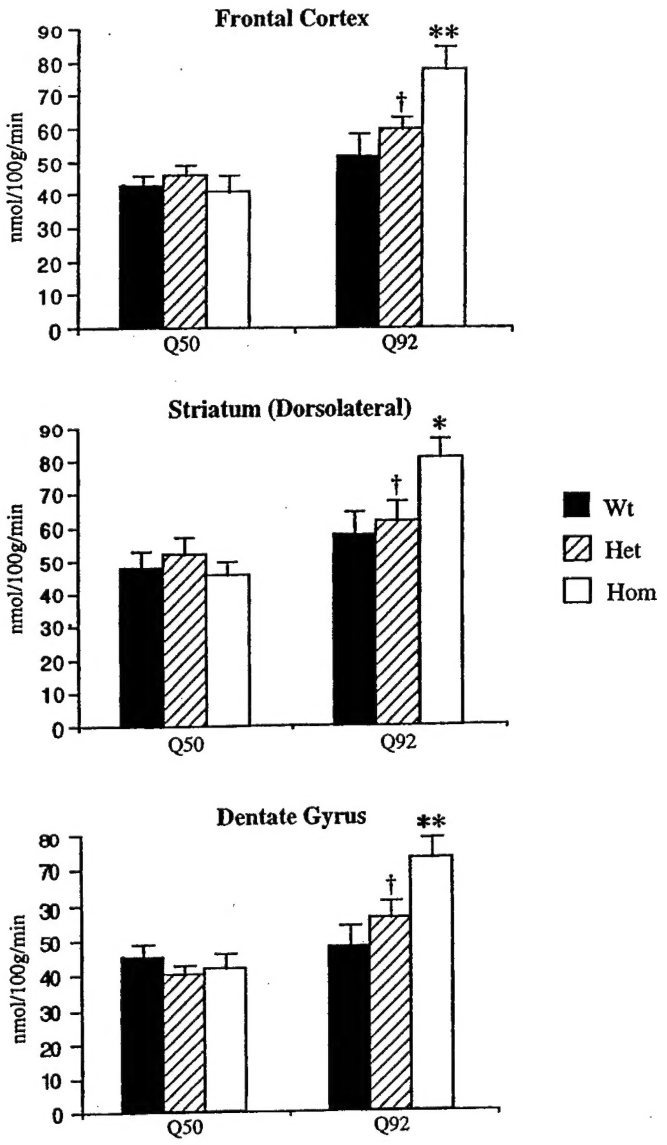

Figure 1: Cerebral Glucose Use in Representative Regions From Hdh^{Q50} and Hdh^{Q92} Mice

$lCMR_{glc}$ (nmol/100g/min) in 4 month-old Hdh^{Q50} and Hdh^{Q92} homozygous (Hom) and heterozygote (Het) mice, and wild-type (Wt, 7/7) littermate controls. Data presented as mean ± SEM, (n=4-6 per group).
* $P < 0.05$, ** $P < 0.01$ relative to levels in 7/7 mice; † $P < 0.05$ relative to 90/7 mice (ANOVA, followed by Fisher's PLSD).

Discussion: We previously found that glucose use rates did not significantly differ between Hdh^{Q50} and Hdh^{Q7} mice in any of the brain regions examined ($p > 0.05$; ANOVA, followed by Fisher's PLSD), and there was no effect of gene dosage in Hdh^{Q50} mice (48/48 vs 48/7). In this study, we found marked increases in glucose use throughout the brains of 90/90 Hdh^{Q92} mice. Increases were significant in 15 of the 21 regions examined. Furthermore, glucose use levels in 90/90 mice were significantly elevated over levels in 90/7 mice in 3 regions, suggesting a potential gene dosage effect, as well as a CAG length-dependent effect (see Figure 1). Recent observations suggest that the rate and extent of huntingtin protein translocation from cytosol to the nucleus, and the formation of nuclear

huntingtin aggregates, increase with higher CAG repeat lengths (Wheeler et al., 2000). Therefore, there is reason to suppose that any energetic changes associated with the huntingtin mutation may also vary in time of onset and extent according to the length of the CAG repeat. We are currently investigating this hypothesis in Hdh^{Q111} mice. The observation that glucose uptake is increased in multiple forebrain regions as a consequence of the gene mutation in Hdh^{Q92} mice may at first seem counter-intuitive. We hypothesize that this reflects increased glucose uptake in an attempt to drive metabolism and overcome a metabolic stress in these animals. The fact that Hdh^{Q92} mice do not develop overt signs of motor dysfunction in their lifetime suggests that in this case the enhanced glucose uptake is sufficient to maintain required ATP production (however, Hdh^{Q92} mice do develop nuclear huntingtin aggregates by 15 months of age, Wheeler et al., 2000). The exact nature of any metabolic stress (eg. glycolytic or mitochondrial) has yet to be elucidated. In contrast, Hdh^{Q111} mice develop motor abnormalities by approximately 19 months of age. It will be interesting to ascertain the time course and magnitude of any metabolic abnormalities in these animals.

Objective #2: **To determine if glucose use is altered over the life-span of a transgenic HD mouse model expressing a human mutant HD fragment: R6/2 HD mice.**

In the original grant protocol we proposed measuring cerebral glucose utilization throughout the brains of HD transgenic R6/2 mice, using the [^{14}C]-2-deoxyglucose *in vivo* autoradiography procedure. We proposed that glucose use measurements both prior to and after symptom onset in these mice (at around 8 weeks) would shed light on whether energetic defects play a causative role in disease pathogenesis. Subsequent to award of this grant, we found that R6/2 mutant mice develop a diabetic profile from approximately 7 weeks of age. Mice show elevated basal blood plasma glucose levels (>> 300mg/dL, relative to 150 mg/dL in physiologically normal mice), and reduced glucose tolerance.

Elevated baseline plasma levels, outside of a range considered physiologically "normal" (5-16mM / 91-290mg/dL in rodents, Sokoloff et al., 1977), confounds use of the [^{14}C]-2-deoxyglucose technique. This *in vivo* imaging procedure utilizes an 'operational equation' to extrapolate rates of glucose utilization in a given brain region from measured parameters, which include: levels of circulating arterial plasma glucose; rate of uptake of injected tracer [^{14}C]-2-deoxyglucose from the circulating blood; and detected levels of [^{14}C] in the brain region of interest at the end of the experiment (Sokoloff et al., 1977). The equation also relies on assumed kinetic rate constants for blood-brain transfer of glucose and 2-deoxyglucose, which are based on measured levels in rodents under physiologically "normal" conditions. Outside of these conditions, the kinetic constants used in the operational equation no longer apply and any extrapolated results become unreliable. Consequently, we have been unable to perform glucose use studies in R6/2 mice due to their hyperglycemic condition, which prevents reliable data being obtained. We investigated measuring glucose use levels in mice before they develop the

diabetic profile (4-6 weeks of age), but studies in these young mice are confounded by their small size, which makes surgical preparation of the animals extremely difficult and increases stress in the mice.

In lieu of these studies in R6/2 mice, we employed another mutant mouse model of HD, N171 mice co-expressing an N-terminal fragment of human mutant huntingtin with an 82-polyglutamine expansion (Schilling et al., 1999). We carried out studies to identify whether metabolic abnormalities are present in mutant N171-82Q mice at 2 months of age, prior to huntingtin aggregate formation, striatal cell loss, behavioral and diabetic phenotype in this model. Results are discussed briefly below.

[^{14}C]-2-Deoxyglucose Measures of Cerebral Glucose Use in N171-82Q HD Mice.
N171-82Q mice develop a relatively rapid disease phenotype characterized by onset of motor abnormalities, nuclear huntingtin aggregate (NII) deposition, and striatal-specific neuronal loss by 3-4 months of age. Mice generally live 4-5 months, and develop a diabetic profile by 3.5-4 months of age. We measured cerebral glucose use rates in conscious N171-82Q mutants, N171-18Q transgenic control mice, and non-transgenic wild-type littermate mice at 2 months of age. This time point was chosen to determine if energetic changes play an early role in the disease process, preceding pathological changes and the onset of diabetes in N171-82Q mice,. Results further implicate cerebral glucose metabolic changes in the pathogenesis in these mutant mouse models of HD.

Physiological Variables: Mice require 1.5-2h for anesthetic clearance from the brain after surgery. Wild type control and N171-18Q mouse basal arterial glucose levels were normal by 2h after surgery (~150 mg/dL). In contrast, N171-82Q mice required 4-5 hours recovery after anesthesia for their basal glucose levels to return from elevated to approaching normal levels. This is likely due to an inability to buffer anesthesia stress-induced glucose upregulation, although mice are not overtly diabetic at this time point. At the time of the experiment, glucose values in N171-82Q mice were elevated relative to control levels (194 ± 10 vs 149 ± 9 mg/dL), but were within the physiologic "normal" constraints of the procedure. Arterial plasma levels did not alter over the time course of the procedure, indicating that mice were not stressed by the technique. Arterial pO2, pCO2 and pH values measured at the end of the 45min 2-DG procedure did not differ between groups and were well within "normal" physiologic ranges for mice.

Local Rates of Cerebral Glucose Use ($lCMR_{glc}$): Local cerebral rates of glucose use in N171-82Q mutant mice show *trends towards increase* in some forebrain regions, relative to levels in wildtype and N171-18Q transgenic control mice, as shown in Tables 2, 3 and 4. Increases in glucose use in 82Q mice reached statistical significance only in frontal cortex (layer IV). Although few alterations reached statistical significance, increases in 82Q mice are marked in a number of regions, not restricted to the striatum. There are also suggestions of differences between the wild type background strain and

N171-18Q mice in some regions including pre-frontal and frontal cortex. Experiments are currently being extended to larger group sizes.

Table 2: CORTICAL REGIONS

Region	Wildtype	N171-18Q	N171-82Q
Pre-Frontal: Layers I-III	55.6 ± 8.8	67.5 ± 2.1	71.7 ± 2.7
Pre-Frontal: Layer IV	59.2 ± 10.0	74.2 ± 1.5	77.2 ± 0.6
Pre-Frontal: Layers V-VI	49.7 ± 5.0	59.2 ± 1.4	62.2 ± 4.4
Frontal: Layers I-III	59.8 ± 7.7	65.5 ± 0.5	68.0 ± 7.9
Frontal: Layer IV	59.5 ± 6.3	70.1 ± 1.1	87.5 ± 2.2*†
Frontal: Layers V-VI	50.9 ± 4.4	54.7 ± 0.9	64.9 ± 3.8
Parietal: Layers I-III	67.0 ± 6.8	71.1 ± 9.2	71.4 ± 3.8
Parietal: Layer IV	73.4 ± 9.3	91.2 ± 15.2	81.4 ± 2.6
Parietal: Layers V-VI	65.9 ± 8.7	74.7 ± 12.7	69.9 ± 2.6
Anterior cingulate	65.6 ± 10.9	77.4 ± 0.8	85.6 ± 2.7
Posterior cingulate	74.0 ± 5.6	102.0 ± 1.6	99.9 ± 9.7

Table 3: LIMBIC AND EXTRAPYRAMIDAL REGIONS

Region	Wildtype	N171-18Q	N171-82Q
Striatum:			
Dorsomedial	61.3 ± 7.2	76.1 ± 6.2	66.9 ± 6.7
Dorsolateral	68.8 ± 7.3	84.6 ± 2.1	81.7 ± 4.1
Ventromedial	64.3 ± 5.4	75.2 ± 0.4	77.6 ± 3.6
Globus Pallidus	37.2 ± 3.5	51.5 ± 5.1	49.8 ± 0.2
Hippocampus:			
CA1	31.9 ± 5.6	33.9 ± 0.7	43.0 ± 0.4
CA3	40.4 ± 6.2	48.4 ± 5.2	52.2 ± 0.1
Dentate gyrus	50.8 ± 7.4	55.2 ± 1.7	62.5 ± 4.3
Lateral habenular nucleus	48.7 ± 9.4	67.4 ± 9.5	67.3 ± 7.0
Subthalamic nucleus	77.0 ± 9.0	89.0 ± 10.0	70.2 ± 2.4
Mammilary body	94.0 ± 22.8	102.5 ± 0.5	99.8 ± 14.3
Substantia nigra:			
pars compacta	59.0 ± 6.6	51.6 ± 3.7	66.0 ± 3.3
pars reticulata	44.6 ± 5.8	42.4 ± 4.9	44.5 ± 2.5
Cerebellar nucleus	87.0 ± 4.5	97.8 ± 4.2	115.5 ± 6.6
Cerebellar hemisphere			
granular layer	29.9 ± 3.1	33.2 ± 3.1	35.9 ± 0.8
molecular layer	41.5 ± 4.6	42.7 ± 1.0	51.5 ± 0.7
Inferior olive	75.3 ± 12.8	68.1 ± 4.7	75.1 ± 2.1
Cerebellar white matter	24.8 ± 4.3	22.2 ± 3.7	29.9 ± 0.4

Table 4: AUDITORY AND VISUAL REGIONS

Region	Wildtype	N171-18Q	N171-82Q
Visual Regions			

Visual Cortex: Layer IV	57.1 ± 3.5	62.2 ± 2.1	68.6 ± 11.9
Lateral geniculate body	57.0 ± 7.5	57.6 ± 1.5	62.2 ± 5.8
Superior colliculus:			
Superficial layer	54.2 ± 7.9	61.2 ± 13.0	61.2 ± 4.4
Deep layer	54.9 ± 6.9	55.8 ± 1.1	63.9 ± 2.9
Auditory Regions			
Auditory Cortex: Layer IV	102.5 ± 4.1	100.6 ± 11.9	121.5 ± 17.7
Inferior colliculus	145.3 ± 13.6	129.1 ± 6.0	164.5 ± 37.3
Superior olive	90.0 ± 6.6	91.0 ± 4.8	108.3 ± 16.2
Medial geniculate body	81.3 ± 6.5	80.7 ± 4.3	92.5 ± 9.3
Cochlear nucleus	87.8 ± 11.1	93.9 ± 6.2	118.9 ± 10.1

Local cerebral metabolic rates for glucose (nmol/100g/min) in heterozygous N171-82Q mutant mice, compared with rates in wild-type littermate controls, and in heterozygous N171-18Q tg controls (n = 3-6 per group).
*$p < 0.05$, relative to levels in wt mice; † $p < 0.05$ relative to levels in 18Q mice (ANOVA & Fischer's PLSD).

(iii) *Discussion:* Results suggest 2 month-old N171-82Q may be beginning to exhibit metabolic changes before phenotype onset. Experiments in older mice will determine if this phenomenon is exacerbated over life-span. Observed increases in glucose use in 82Q mice suggest potential metabolic defects preceding behavioral and pathological changes in the N171-82Q HD mouse model. Changes are reminiscent of those observed in *Hdh* knock-in mice. Increased glucose use may reflect a compensatory effort by cells to overcome a metabolic insult by increasing glycolytic generation of ATP, or increase substrate influx into mitochondria.

Objective #3: **Measurement of electron transport chain enzyme activities in R6/2 HD mice, before and after symptom onset and pathological changes.**

There are multiple reports of metabolic enzyme dysfunction in HD post-mortem brain, notably reduced complex II-III, IV, and aconitase activities localized to caudate and putamen. We used spectrophotometric assays to measure the activities of enzyme components of the glucose metabolic pathways, in whole forebrain and cerebellum homogenates from R6/2 mice at 3.5, 8 and 12 weeks of age. Homogenates were prepared from frozen brain tissue. Enzyme activities measured were glyceraldehyde-3-phosphate dehydrogenase (GAPDH; glycolytic enzyme); aconitase and citrate synthase (Kreb's cycle enzymes); complexes I, II-III and IV of the mitochondrial electron transport chain. Complex enzyme activities (per mg protein) were corrected by the citrate synthase activity (per mg protein; a mitochondrial matrix enzyme) as a correction for differential mitochondrial number between preparations. Results are shown in Table 5. No alterations in activities of any of the enzymes measured were detected in whole brain homogenates from 3.5 and 8 week-old mice. Increased complex I and II-III activities were detected in R6/2 forebrain at 12 weeks of age. GAPDH activity was significantly elevated in the cerebellum of 12 week-old R6/2 mice relative to wild type littermate control levels. Aconitase activity was unaltered in all regions at all time-points (data not shown).

Table 5: Metabolic enzyme activites in R6/2 HD mutant mice.

		FOREBRAIN		CEREBELLUM	
		Wt	R6/2	Wt	R6/2
Complex I: (/CS)	3 wk	0.14 ± 0.02	0.16 ± 0.01	0.17 ± 0.03	0.13 ± 0.03
	8 wk	0.11 ± 0.01	0.12 ± 0.02	0.13 ± 0.02	0.14 ± 0.02
	12 wk	0.10 ± 0.01	0.14 ± 0.02 *	0.15 ± 0.02	0.16 ± 0.02
Complex II-III: (/CS)	3 wk	0.19 ± 0.02	0.19 ± 0.03	0.20 ± 0.03	0.20 ± 0.02
	8 wk	0.13 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	0.16 ± 0.03
	12 wk	0.13 ± 0.01	0.18 ± 0.02 *	0.15 ± 0.01	0.14 ± 0.01
Complex IV: (/CS)	3 wk	1.24 ± 0.21	1.46 ± 0.16	1.26 ± 0.17	1.35 ± 0.13
	8 wk	0.88 ± 0.16	0.85 ± 0.13	0.85 ± 0.09	0.98 ± 0.29
	12 wk	1.09 ± 0.17	1.25 ± 0.16	1.20 ± 0.22	1.13 ± 0.13
GAPDH: (/mg prot.)	3 wk	1.26 ± 0.06	1.45 ± 0.14	1.24 ± 0.14	1.26 ± 0.10
	8 wk	2.84 ± 0.22	2.61 ± 0.16	1.72 ± 0.26	1.68 ± 0.21
	12 wk	2.67 ± 0.30	3.12 ± 0.20	1.91 ± 0.21	2.65 ± 0.33 *

Data are mean ± SEM enzyme activities in mice 3, 8 (n = 5-6 per group) and 12 (n = 10 per group) weeks of age. Complex activities (nmol/min/mg/protein) corrected for citrate synthase (CS) activity / mg protein. * $p < 0.05$, significant difference relative relative to age-matched Wt mice (ANOVA and post-hoc Fisher PLSD).

Discussion: Activities of all enzymes measured were unaltered in both forebrain and cerebellum homogenate preparations before symptom onset (3.5 and 8 weeks of age). Increases in complex I and II-III activities in forebrain, and GAPDH in cerebellum, were evident in symptomatic 12 week-old mice. Our findings differ from previously reported enzyme activities in R6/2 12 week-old mice, which show reduced complex II-III activity and markedly decreased aconitase activity in forebrain preparations (Tabrizi et al., 2000). Reasons for these discrepancies are not obvious, but one possibility may be our use of frozen tissue to generate homogenate preparations (stored for 6-12 months) rather than fresh tissue. Our results also do not correlate with findings of reduced complex II-III and IV, and aconitase activities in affected brain regions (caudate and putamen) of symptomatic, end-stage HD patients post-mortem brain (Browne et al., 1997; Gu et al., 1997; Tabrizi et al., 1999). This may indicate that the pathophysiology of the R6/2 model differs from HD pathogenesis in humans, although this is not supported by the findings of Tabrizi and colleagues (2000). Alternatively, region-specific alterations in brain regions vulnerable to damage in HD (ie. the caudate and putamen in human, analagous to striatum in mouse) may be masked by the use of whole forebrain homogenate preparations in this study. Therefore, we are currently performing enzyme assays in striatal, cortical and cerebellar tissue preparations. These studies are not yet complete, due to slow breeding of the large number of animals required to generate sufficient tissue for these studies.

Objective #4. **NMR assessment of cerebral lactate levels in *Hdh* and R6/2 mice.**

Increased lactate production is a marker for metabolic dysfunction, due to elevated glycolytic activity or pyruvate shunt to form lactate rather than enter mitochondrial metabolic pathways. Lactate levels in brain can be measured non-invasively *in vivo* by NMR spectroscopy in anesthetized mice. We have carried out NMR spectroscopic measurements in the brains of R6/2 and $HdhQ^{92}$ mice, at multiple time-points over their life-spans. Results of these studies are currently under analysis, and will be presented in detail in the next report.

7. KEY RESEARCH ACCOMPLISHMENTS

1. The finding that cerebral glucose use is significantly increased in *Hdh*^{Q92} CAG knock-in mice at 4 months of age, relative to levels in wild type animals, and relative to levels in *Hdh*^{Q50}mice (Year 1 results).

2. The finding that cerebral glucose use in *Hdh*^{Q92} CAG knock-in mice shows a gene-dosage effect, with homozygote (90/90 CAG repeat) mice showing increased glucose use elevations than heterozygote (90/7) mice. In fact, the magnitude of glucose use increases in 90/90 mice is approximately double levels in 90/7 mice.

3. The finding that alterations in cerebral glucose use in *Hdh*^{Q92} mice is evident at 4 months of age in these animals (the earliest time-point investigated). These changes precede pathological or behavioral changes in *Hdh*^{Q92} mice. (NB: *Hdh*^{Q92} mice do not develop a movement disorder, unlike *Hdh*^{111} mice and other HD transgenic mouse models). There is evidence that mutant huntingtin protein (*htt*) is translocating to the nucleus at this time-point, but neuronal intranuclear inclusions (NII) are not evident until 15 months of age in this model (Wheeler et al., 2000). Aggregate formation occurs faster in *Hdh*^{Q111} mice, hence we are currently examining the same parameters of energy metabolism in these animals.

4. The finding that presymptomatic increases in glucose utilization also occur in multiple forebrain regions in another transgenic HD mouse line, N171-82Q mice expressing a mutant human HD fragment with an 82 polyglutamine repeat. Taken together, findings suggest that metabolic compromise may be an early event in the pathophysiology associated with the expression of mutant huntingtin protein. Glucose use elevations suggest that cells may be attempting to increase glycolytic ATP production, or increase substrate feed into mitochondrial energetic pathways to compensate for a metabolic stress or blockade. The exact mechanism has yet to be elucidated.

5. The finding that R6/2 transgenic HD mice develop a diabetic profile, with onset at 7-8 weeks of age (around the time of movement disorder symptom onset).

6. Observations that metabolic enzymes which show impaired activity in late-stage HD patients (reduced complex II-III and IV in post-mortem brain) do not show evidence of altered activities in pre-symptomatic R6/2 HD mouse brains (whole tissue homogenate preparations; 3.5 and 8 week-old mice). Some alterations are evident in late-stage (12 week-old) R6/2 mice. However, the nature of the metabolic enzyme activities we detected did not correlate with observations in symptomatic HD patients (Browne et al., 1997), or with previously reported alterations in homogenate samples from 12 week old R6/2 mice (Tabrizi et al., 2000). It is possible that use of whole brain homogenate preparations is masking any subtle region-specific changes occur, for example in the striatum of R6/2 mouse brains. Therefore we are currently repeating these assays in striatal preparations from these mice.

8. REPORTABLE OUTCOMES

Browne SE, Beal MF. Huntington's disease. In: Funtional Neurobiology of Ageing. PR Hof, CV Mobbs, Eds. *Academic Press* (2000) 711-725

Browne SE, DiMauro J-P P, Narr S. Metabolic changes precede phenotypic changes in mutant mouse models of HD. *World Federation on Neurology Research Group on Huntington's Disease* (2001) *In press.*

Browne SE, Yang L, Fuller SW, Beal MF. Metabolic changes precede pathologic changes in the G93A mouse model of a familial amyotrophic lateral sclerosis. *Soc. Neurosci. Abs.* (2001) *In press*

J-P P DiMauro, S Narr, MF Beal, **Browne SE**. Cerebral energy metabolism in mutant mouse models of Huntington's disease. *Soc. Neurosci. Abs.* (2001) *In press*

Browne SE, Wheeler V, White JK, Fuller SW, MacDonald, M, Beal MF. Dose-dependent alterations in local cerebral glucose use associated with the huntingtin mutation in *Hdh* CAG knock-in transgenic mice. *Soc. Neurosci. Abs.* (1999) 25: 218.11.

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9. CONCLUSIONS

We have made substantial progress in characterizing the nature of changes in cerebral energy metabolism seen in the *Hdh* knock-in mouse model of HD *in vivo*. Outcomes suggest that expression of mutant expanded CAG repeats in the *HD* gene induces energetic defects. Glucose use changes are dependent on CAG repeat length and gene dosage in *Hdh* mice. Moreover, metabolic changes occur before the onset of NII formation or symptom onset. Preliminary studies in the N171-82Q HD transgenic mouse model also show evidence of pre-symptomatic metabolic alterations. The exact cause and effects of altered glucose metabolism in HD mouse models are yet to be elucidated, but findings imply that energetic abnormalities may play an intrinsic role in the pathogenic process triggered by the HD gene mutation. Further investigations of temporal progressions of alterations associated with the gene mutation are underway. We are also pursuing determinations of metabolic enzyme activities in specific brain regions of mutant HD mice, and are currently analyzing data from NMR measures of lactate generation in HD mouse models. Results to date suggest that the experimental paradigms employed in this project may represent model systems for testing the efficacies of putative metabolism-enhancing treatments as HD therapeutics.

Projects planned for Year 3 of this grant are:

1) Measurement of cerebral levels of oxidative damage markers (protein carbonyls; nDNA OH^8dG; hydroxyl radical production; nitrotyrosine levels) in *Hdh* knock-in and R6/2 HD Tg mouse models.
2) Measurements of oxidative damage markers in G93A FALS Tg mice.
3) HPLC measurement of cerebral energy metabolite levels in HD *Hdh* and R6/2 Tg mice.
4) Measurement of $lCMR_{glc}$ in a rat model of 3-nitropropionic acid neurotoxicity.

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11 APPENDIX

Copy: Browne SE, Beal MF. Huntington's disease. In: Funtional Neurobiology of Ageing. PR Hof, CV Mobbs, Eds. *Academic Press* (2000) 711-725

Susan E. Browne and M. Flint Beal

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Huntington's Disease

Huntington's disease is one of several devastating neurodegenerative diseases, including amyotrophic lateral sclerosis and Alzheimer's disease (AD), which are characterized by late onset in adult life. Their debilitating symptoms include cognitive dysfunction, behavioral changes and mood disturbances, and movement disorders, and in the cases of amyotrophic lateral sclerosis and Huntington's disease paralysis and death can occur. As the mean age of the population increases, so does the frequency of occurrence of these disorders. Symptoms are associated with region-specific loss of neurons within the central nervous system (CNS), but to date the mechanism of this selective neuronal death remains unknown. Several different etiological processes may play roles, and strong evidence from studies in humans and in animal models suggests the involvement of energy metabolism dysfunction, excitotoxic processes, and oxidative stress. The recent development of transgenic mouse models expressing the human Huntington's disease mutation has provided novel opportunities to determine the chronological order of events underlying the selective neuronal death seen in the disease, which have hitherto been impossible to determine in humans.

I. Introduction

Over a century elapsed between the first description of the Huntington's disease phenotype by George Huntington in 1872 and the discovery of the genetic defect underlying the disease in 1993 (Huntington's Disease Collaborative Research Group, 1993). A physician in Long Island, New York, the familial inheritance of the disease was well known to Huntington from the family histories passed down to him by his grandfather and father. Huntington's disease is now known to be an autosomal dominantly inherited neurodegenerative disorder characterized by the adult onset and progressive development of behavioral abnormalities, cognitive impairment, and involuntary choreiform movements, with a typical duration of 15–20 years. The genetic abnormality in Huntington's disease is a CAG repeat expansion in a gene encoding a 350 kDa protein of unknown function, termed "huntingtin" (Huntington's Disease Collaborative Research Group, 1993). It is thought to be a true dominant disorder since homozygous patients do not seem to differ from heterozygote carriers in either age of onset, duration, or severity of the disease (Durr *et al.*, 1999). Despite its identification, the definitive role of mutant huntingtin in neuronal degeneration remains unknown. As discussed below, the insidious progression of motor and behavioral disturbances in Huntington's disease reflects the selective pattern of cell loss in the brain and the specific neurotransmitter pathways affected. The reason for the preferential vulnerability of striatal neurons in Huntington's disease is still enigmatic, and cannot be simply explained in terms of the distribution of abnormal huntingtin since the gene mutation is expressed throughout the body. However, experimental evidence suggests that the pathogenesis of cell death in Huntington's disease is linked to a gain of function of mutant huntingtin. In addition, studies in both human Huntington's disease brain and transgenic mouse model of Huntington's disease have identified widespread neuronal intranuclear and cytoplasmic aggregations of mutant huntingtin that may be linked to the disease process. In the following review we have collated the experimental information available to date to give insight into the link between the Huntington's disease gene mutation and selective neuronal dysfunction and death in this disease.

II. Neuropathological Features and Motor Dysfunction in Huntington's Disease

A. Pathological Changes in Huntington's Disease Brain

The typical neuropathological features of Huntington's disease are progressive caudal to rostral degeneration of the caudate-putamen (Vonsattel and DiFiglia, 1998). Patients are graded at postmortem according to the extent of gross and microscopic measures of neuropathological severity, grades ranging from 0 to 4 with increasing severity and extent of striatal involvement (Vonsattel *et al.*, 1985). Briefly, grade 0 brains exhibit 30–40% neuronal loss in the head of the caudate, with no visible signs of reactive gliosis. In contrast, in grade 4 more than 95% of striatal neurons are lost, the striatum is severely atrophic with marked gliosis, and about 50% of end-stage

cases show cell loss in the nucleus accumbens. Most Huntington's disease cases reach grade 3 or 4 by the time of death, by which stage nonstriatal regions are also involved, in particular the globus pallidus, cortex, and, to a lesser extent, thalamus, subthalamic nucleus, substantia nigra, white matter, and cerebellum (Sotrel *et al.*, 1991; Braak and Braak, 1992; Vonsattel and DiFiglia, 1998). Fibrillary astrogliosis occurs in the striatum, but has not been reported in other affected areas, and no inflammatory responses are involved (Myers *et al.*, 1991). In cases of juvenile onset Huntington's disease cerebellar atrophy is particularly prevalent.

The striatal cells most susceptible to degeneration in Huntington's disease are medium spiny projection neurons (Beal, 1994a). Spiny neurons, which constitute 80% of striatal neurons, are the principal input and output neurons of the striatum. All contain the inhibitory neurotransmitter γ-aminobutyric acid (GABA), while subsets also contain enkephalin (ENK), Substance P (SP), dynorphin, or calbindin. The other major class of striatal neurons are aspiny interneurons. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), neuropeptide Y (NPY), somatostatin, and nitric oxide synthase (NOS) typically colocalize in medium-sized aspiny neurons, and some also contain cholecystokinin or the calcium-binding protein parvalbumin. In Huntington's disease striatum, spiny projection neurons containing SP or ENK degenerate earliest in the course of the disease, whereas aspiny interneurons and the larger cholinergic interneurons are relatively spared (Ferrante *et al.*, 1987; Beal *et al.*, 1988). There is also some hierarchy in the vulnerability of different spiny neuron subpopulations; ENK-immunoreactive neurons projecting to the external segment of the globus pallidus (GPe) degenerate prior to SP-containing neurons projecting to the internal segment (GPi) (Reiner *et al.*, 1988; Richfield *et al.*, 1995; Sapp *et al.*, 1995). Spiny neurons also undergo morphological changes in the course of the disease process in Huntington's disease, including recurving of the dendrites, altered shape and size of the spines, and increased density of spines.

Surviving neostriatal neurons are generally morphologically normal, although some are reduced in size and contain elevated levels of the oxidative damage marker lipofuscin. A subpopulation of "neostriatal dark neurons" has also been described by Vonsattel *et al.* (1985), scattered between the zones of atrophic and healthy cells. Interestingly, markers of apoptotic cell death have been detected in these neostriatal dark neurons (for example, granulation of the cytoplasm and condensation of nuclear chromatin and labeling by TdT-mediated dUTP-biotin nick end-labelling [TUNEL]; Vonsattel and DiFiglia, 1998).

B. Motor Dysfunction

The motor defects typical of Huntington's disease result from the disruption of basal ganglia-thalamocortical pathways which regulate movement control. The neostriatum (caudate nucleus and putamen) receives excitatory glutamatergic inputs from the entire neocortex, the first step in the anatomical loop responsible for the initiation and execution of movement. Processed signals are transmitted via basal ganglia output nuclei (GPi, the substantia nigra pars reticulata, SNr, and ventral pallidum) to the thalamus, which in turn sends excitatory projections to areas of the frontal cortex associated with motor planning and execution (Albin *et al.*, 1989; Alexander and Crutcher, 1990; Graybiel *et al.*, 1994). The GABAergic basal ganglia output projections to the thalamus maintain a tonic inhibition of their target nuclei, which is modulated by two opposing pathways (direct and indirect) which integrate the input and output compartments within the basal ganglia (Fig. 49.1). It is an imbalance in the relative contributions of these two regulatory pathways that triggers, and dictates the nature of, the motor dysfunction in Huntington's disease. In the direct (monosynaptic) pathway, activation of striatal efferents containing GABA and SP projecting directly to the GPi results in disinhibition of thalamic activity. In contrast, in the indirect (polysynaptic) pathway striatal efferents containing GABA and enkephalin project to the GPe which sends purely GABAergic projections to the subthalamic nucleus. From here, excitatory efferents (probably glutamatergic) project to the basal ganglia output nuclei (SNr and GPi). The GPe projection generally exerts a tonic inhibition on the subthalamic nucleus. Activation of GABA/ENKergic striatal efferents tends to suppress activation of GPe neurons, causing disinhibiton of the subthalamic nucleus and hence an increase in the excitatory innervation of the basal ganglia output nuclei. This leads to an increased inhibitory input to the target thalamic nuclei. Thus, cortical function is differentially modulated depending on which basal ganglia pathway, and therefore which thalamocortical pathway, is activated (Albin *et al.*, 1989; Alexander and Crutcher, 1990). In Huntington's disease there is preferential loss of the GABA/ENK-containing neurons comprising the indirect pathway. "Disinhibition" of the thalamus results, which is manifest in Huntington's disease patients by the development of involuntary choreic movements. The later onset of a rigid akinetic state in some Huntington's disease patients is thought to result from the additional loss of striatal GABA/SP-containing efferents projecting directly to the GPi (Albin *et al.*, 1990).

III. Mutant Huntingtin Protein in Huntington's Disease

The genetic defect in Huntington's disease is an expansion of an unstable CAG repeat encoding polyglutamines (Q_n) at the 5′ end of a gene on chromosome 4, *IT15* ("interesting transcript 15"), now termed *huntingtin (HD)* (Huntington's Disease Collaborative Research Group, 1993). Similar trinucleotide mutations in different genes are responsible for at least seven other neurodegenerative disorders: the spinocerebellar ataxias (SCA1, SCA2, SCA3 [Machado-Joseph disease], SCA6, and SCA7), dentatorubral-pallidoluysian atrophy, and spinal bulbar muscular atrophy (Ross, 1995; Zoghbi, 1997). While the genes affected in these disorders have now been identified, only in spinal bulbar muscular atrophy is the function of the affected protein known (androgen receptor). All the glutamine repeat diseases involve neuronal loss and gliosis. However, while gene expression is widespread throughout the body, cell death occurs in specific regions of the brain and spinal cord. How these genetic defects lead to progressive, selective neurodegeneration, remains elusive. The gene product in Huntington's disease, huntingtin protein, is a 348 kDa protein containing 3144 amino acids. Normal, unaffected indi-

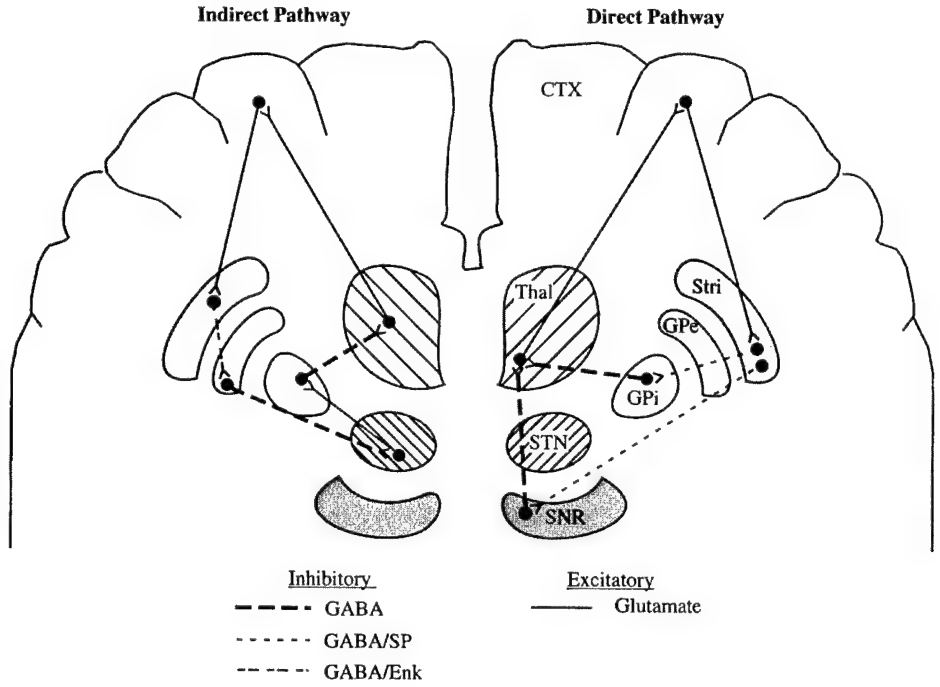

FIG. 49.1. Corticofugal pathways controlling movement. A representative diagram showing the major neurotransmitter systems involved in the direct (right) and indirect (left) basal ganglia output pathways. Activation of the indirect pathway increases the inhibitory input to thalamic nuclei, resulting in reduced excitatory output to the cortex. In contrast, activation of GABA/SP-containing striatal efferents in the direct pathway results in inhibition of the GPi and SNR projection to the thalamus, effectively releasing the thalamus from pallidal inhibition. As a result, the excitatory output to the cortex increases. Choreic movements in Huntington's disease are thought to result from reduced activity of the indirect pathway, due to loss of GABA/Enkephalinergic neurons (Albin *et al.*, 1989; Andrews and Brooks, 1998). CTX, cerebral cortex; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; Thal, Thalamus; SNR, substantia nigra pars reticulata; STN, subthalamic nucleus; Stri, striatum.

viduals typically have trinucleotide repeats of 11–34 CAGs. Expansion to 34–39 CAG repeats in one or both alleles confers the possibility of developing Huntington's disease, while the disease is inescapable when CAG repeats in either allele exceed 39. The trinucleotide repeat is polymorphic and undergoes alterations during meiosis, generally fluctuating by ± 1–5 repeats per transmission, although larger increases can occur following paternal transmission (Ross, 1995). The physiological functions of both normal and mutant huntingtin have not yet been determined, although several features of the Huntington's disease phenotype are known to be influenced by CAG repeat length, such as age of onset of the disease (Andrew *et al.*, 1993; Duyao *et al.*, 1993; MacDonald *et al.*, 1999) and the extent of DNA fragmentation in Huntington's disease striatal neurons (Butterworth *et al.*, 1998). CAG repeat length has also been correlated with neuropathological severity, although this observation is controversial since the grade of disease at time of death is dependent on a number of factors also influenced by repeat length, including age of onset and disease duration (Furtado *et al.*, 1996; Sieradzan and Mann, 1998).

Distribution studies give little insight into the involvement of mutant huntingtin in the regional selectivity of cell loss in the disease, since huntingtin protein is ubiquitously expressed throughout the body. The fact that its distribution shows no apparent selectivity for cerebral regions targeted by the disease process suggests that another property of basal ganglia neurons confers vulnerability to degeneration in Huntington's disease (Strong *et al.*, 1993; Sharp *et al.*, 1995). However, recent immunohistochemical studies suggest that huntingtin may be differentially distributed at the cellular level within the striatum. Ferrante *et al.* (1997) reported that within the striatum huntingtin immunoreactivity is heterogeneous, the patch compartment showing low levels or no immunoreactivity in neurons and neuropil, while levels are relatively higher in the matrix. Double labeling techniques revealed higher levels of huntingtin expression in medium spiny neurons and colocalization with calbindin, in contrast to little or no colocaliztion of huntingtin with NADPH-d or NOS neurons, suggesting that there is some correlation between huntingtin's cellular location and cell vulnerability. A more recent study (Fusco *et al.*, 1999) supports Ferrante and colleagues' observations that huntingtin protein load varies within the striatal population of medium-sized neurons and is not consistently found in all medium spiny projection neurons. Taken with their observations that large striatal cholinergic interneurons which are relatively spared in the disease contain high levels of huntingtin, Fusco and colleagues suggest that the huntingtin mutation is not directly toxic to cells.

At the neuronal level, huntingtin protein is widely expressed throughout cells, with a largely cytoplasmic distribution in perikarya, axons, dendrites, and some nerve terminals, and protein fragments have been identified in neuronal nuclei. Subcellular fractionation studies revealed an association of

huntingtin with synaptic vesicles (DiFiglia *et al.*, 1995), while another report suggests association with the microtubules (Gutekunst *et al.*, 1995), implicating potential roles in intracellular trafficking and synaptic function. N-terminal fragments of huntingtin form ubiquinated protein aggregates in neuronal nuclei (neuronal intranuclear inclusions) and in dystrophic neurites (cytoplasmic inclusions). These protein aggregates have been identified in both Huntington's disease brain and in the brains of transgenic mice expressing a fragment of human mutant huntingtin (Davies *et al.*, 1997; DiFiglia *et al.*, 1997; Reddy *et al.*, 1998; Hodgson *et al.*, 1999). The mechanism of inclusion formation has not yet been detrmined, but Perutz and colleagues (1994) suggest that the expanded polyglutamine stretches in mutant huntingtin lend themselves to the formation of β-pleated sheets held together by hydrogen bonds between amide groups. CAG repeat length appears to be critical for aggregate formation (Scherzinger *et al.*, 1997). Similar intranuclear inclusions also occur in other CAG repeat disorders including spinocerebellar ataxia type 3 (Paulson *et al.*, 1997) and in transgenic mice expressing merely an expanded CAG repeat (Ordway *et al.*, 1997), suggesting that nuclear inclusions are a common product of trinucleotide expansions irrespective of the affected gene.

The toxic function associated with mutated huntingtin appears to be due to gain of a novel function, rather than loss of wild-type huntingtin function, since murine *HD* homolog-null mice die *in utero* while heterozygous knock-out mice show little or no pathology (Duyao *et al.*, 1995). Further, Huntington's disease knock-out mice are completely rescued by crossing into knock-in Hdh^{Q50} mice with a mutant polyglutamine expansion (48 CAGs), while mice expressing abnormally low levels of murine huntingtin exhibit developmental abnormalities (White *et al.*, 1997). Putative mechanisms of huntingtin dysfunction include altered cellular interactions. These include suggestions that expanded glutamine repeats may allow protein–protein interactions or that polyglutamines might be substrates for transglutaminases (Perutz *et al.*, 1994; Cooper *et al.*, 1997b). The principal candidates for protein interactors are huntingtin-associated protein-1 (HAP-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Li *et al.*, 1995; Burke *et al.*, 1996). Others include calmodulin, caspase-3, α-adaptin, cystathionine β-synthase, and huntingtin interacting protein (HIP-1) (see Aronin *et al.*, 1999). Interestingly, in the case of calmodulin the polyglutamine expansion seems to increase huntingtin's affinity (Bao *et al.*, 1996). A potential role for HAP-1 in the pathogenesis of Huntington's disease has largely been discounted on the basis of reports that although it is found only in brain it does not show a preferential striatal distribution, and that HAP-1 does not interact with the mutant stretch in huntingtin (Bertaux *et al.*, 1998). GAPDH is a critical glycolytic enzyme, which led to intriguing proposals that an interaction with mutant huntingtin might impair metabolic function. Arguing against this idea, we found that the glycolytic function of GAPDH is not altered in postmortem Huntington's disease brain tissue (Browne *et al.*, 1997). However, a recent study in fibroblast preparations showed that GAPDH's glycolytic activity is less responsive to metabolic stress in Huntington's disease patients than in fibroblasts from control subjects and unaffected Huntington's disease family members (Cooper *et al.*, 1998). GAPDH also has a number of other functions within the cell which might be altered by an interaction with huntingtin. These include a role as a uracil DNA glycosylase in DNA repair, and binding to a number of proteins including DNA, RNA, ATP, actin, tubulin, amyloid precursor protein, and calcyclin. GAPDH expression is very susceptible to metabolic stress, and a glycolytically inactive form of the enzyme found in the nucleus has been implicated in apoptotic mechanisms in neurons and somatic cells (Saunders *et al.*, 1997; Sawa *et al.*, 1997). The potential for huntingtin to deleteriously affect GAPDH function is supported by a report that both GAPDH and α-ketoglutarate dehydrogenase are inactivated by fusion proteins containing polyglutamine stretches of pathological length, in reactions catalyzed by transglutaminase (Cooper *et al.*, 1997a). Tissue transglutaminases (tTGase) are also implicated in the pathogenesis of trinucleotide repeat disorders including Huntington's disease due to another functional role within neurons and astrocytes, catalyzing Ca^{2+}-dependent cross-linking of glutamine residues with lysine and polyamines in other proteins (Aeschlimann and Paulsson, 1994; Kahlem *et al.*, 1996; Lorand, 1996; Cooper *et al.*, 1997a,b, 1999).

IV. Huntingtin Aggregates: Toxic, Protective, or Inert?

A great deal of debate surrounds the issue of whether neuronal intranuclear inclusion deposition plays a causative role in the pathogenesis of cell death in Huntington's disease, or is merely a secondary event. There is conflicting evidence about the role of neuronal intranuclear inclusions, although at present the weight of opinion seems to favor a lack of involvement, or even a neuroprotective role. The original thesis that nuclear localization of huntingtin aggregates was essential for pathogenic processes came from observations in the R6/2 transgenic mouse line, that neuronal intranuclear inclusion deposition preceded symptoms and pathology in the mouse phenotype (Mangiarini *et al.*, 1996; Davies *et al.*, 1997). Expanded CAG repeats have also been shown to induce huntingtin aggregation and cell death in transfected cerebellar granule cell cultures (Moulder *et al.*, 1998). In addition, transfection of a human mutant huntingtin fragment into *Drosophila* eye cells induced CAG repeat-dependent photoreceptor degeneration and death, putatively via apoptotic mechanisms (Jackson *et al.*, 1998).

The case against a pathogenic role of neuronal intranuclear inclusions is steadily growing. In Huntington's disease postmortem brain, neuronal intranuclear inclusion deposition in the cortex and striatum does not mirror the pattern of cell death in the disease (Ferrante *et al.*, 1997; Fusco *et al.*, 1999). Neuronal intranuclear inclusions and cytoplasmic inclusions are seen in NADPH-d neurons spared in the disease, but are not found in acetylcholinesterase- and choline acetyltransferase-positive interneurons, suggesting that neuronal intranuclear inclusion formation is not critical for cell death mechanisms. Also, in the majority of Huntington's disease patients huntingtin aggregates are most commonly found in neurites, with the exception of 5–10% of patients with juvenile onset of disease in whom neuronal intranuclear inclusions are prevalent in the cortex and striatum (Aronin *et al.*, 1999). In addition, Sawa

et al. (1999) demonstrated in cultured Huntington's disease and control lymphoblasts that stress-induced depolarization is much greater in Huntington's disease mitochondria than in mitochondria from controls, in the absence of any neuronal intranuclear inclusion deposition—suggesting that neuronal intranuclear inclusions are not necessary for cellular dysfunction. They went on to demonstrate that this mitochondrial dysfunction is linked to apoptotic cell death. Furthermore, Saudou and colleagues (1998) recently reported cell-selective neurodegeneration resembling apoptotic cell death in cultured striatal (but not hippocampal) neurons transfected with a human mutant huntingtin fragment, independent of the presence of intranuclear inclusions. Observations that suppression of neuronal intranuclear inclusion deposition resulted in increased cell death in this neuronal population led to the proposal that neuronal intranuclear inclusion deposition may reflect a protective mechanism within cells.

Another study in cultured mouse clonal striatal neurons supports the argument that nuclear aggregates are unnecessary for the cell death mechanism to be activated in Huntington's disease. Kim *et al.* (1999) demonstrated that transfecting mouse clonal cells with either full-length or truncated huntingtin containing mutant CAG repeat lengths induced the formation of both nuclear and cytosolic inclusions, whereas huntingtin with wild-type CAG repeats remained within the cytoplasm. Nuclear inclusions consisted largely of N-terminal cleaved fragments of huntingtin, while cytoplasmic inclusions contained both fragments and intact proteins. Apoptotic features were present in both wild-type and mutant huntingtin transfected cells, but were exacerbated in mutant cells. Findings that inhibiting caspase activity with Z-VAD-FMK increased cell survival, but had no effect on either neuronal intranuclear inclusion or cytoplasmic inclusion number, imply that neuronal death is independent of aggregate formation in this model. This hypothesis was supported by observations that another caspase inhibitor, Z-DEVD-FMK, reduced neuronal intranuclear inclusion and cytoplasmic inclusion number but had no effect on cell survival. The authors also demonstrated that only mutant, and not wild-type, huntingtin underwent cleavage to form N-terminal fragments, which suggests that the polyglutamine domain confers some propensity for cleavage by caspases (Kim *et al.*, 1999). In another study, nuclear import and export sequences were inserted into huntingtin fragments in 293T cultured cells to alter their normal distribution within the cells (Hackam *et al.*, 1999). Results show that toxicity of mutant huntingtin is unaffected by the intracellular location of huntingtin aggregates. Although neither full-length huntingtin or huntingtin fragments have yet been found in mitochondria, an effect on mitochondrial function cannot be ruled out. For instance, huntingtin may play a role in mitochondrial trafficking, or alternatively neuronal intranuclear inclusions may influence nuclear transcription and thus affect the expression of nuclear-encoded proteins including subunits of mitochondrial complex II. The latter is of particular note since complex II activity is impaired in affected brain areas in Huntington's disease.

Transgenic and knock-in mouse models (discussed in section VI) further support the argument that neuronal intranuclear inclusions are not needed for cell toxicity in Huntington's disease models. Although neuronal intranuclear inclusion deposition precedes behavioral symptoms and neurotransmitter changes in R6/2 mice, there is no direct link between the distribution of neuronal intranuclear inclusion deposition and patterns of cell death or dysfunction in several other mutant Huntington's disease mouse models (Reddy *et al.*, 1998; Hodgson *et al.*, 1999; Levine *et al.*, 1999). In addition, R6/2 transgenic mice also develop neuronal inclusions in many postmitotic peripheral tissues from about 6 weeks of age, including both skeletal and cardiac muscle, kidney, liver, pancreas, and adrenal glands (Sathasivam *et al.*, 1999). Peripheral aggregate formation seems to coincide with tissue atrophy, but there is no direct evidence of cell death. It is of interest, therefore, that unlike the brain where the bulk of aggregtes are neuritic, inclusions in skeletal muscle cells are found solely within nuclei. There is also increasing evidence from transgenic mouse models that the mechanism of neuronal death in other polyglutamine repeat disorders is independent of nuclear aggregation of the mutant protein. Lin *et al.* (2000) report that polyglutamine expansion in ataxin-1 causes the downregulation of several neuronal genes involved in signal transduction and calcium homeostasis in SCA-1 mice expressing the transgene. More interestingly, these changes precede any pathological changes in the mice by at least 3 weeks (pathology is evident at 6 weeks). Mutant ataxin-1 also forms intranuclear inclusions in cerebellar Purkinje cells of SCA-1 mice, and in humans, by aggregating with proteosomes and ubiquitin (Cummings *et al.*, 1998). However, these inclusions do not appear to be pathogenic, since decreasing the frequency of nuclear inclusion formation (by treating with E6-AP ubiquitin ligase) actually exacerbated the rate and extent of pathology in SCA-1 mice (Cummings *et al.*, 1999).

V. Putative Mechanisms of Cell Death

The main question still confounding researchers is how the huntingtin mutation results in selective neuronal cell loss in Huntington's disease. The definitive answer is still elusive, but several hypotheses exist.

A. Bioenergetic Defects

One hypothesis is that the gain of function associated with expanded polyglutamine repeats leads either directly or indirectly to a defect in energy metabolism, potentially via secondary excitotoxicity (Albin and Greenamyre, 1992; Beal, 1994b). Reduced ATP production due to impaired energy metabolism can lead to partial cell depolarization by making neurons more vulnerable to endogenous levels of glutamate. The concomitant increase in Ca^{2+} influx into neurons may trigger further free radical production, exacerbating damage to cellular elements. This hypothesis is supported by findings that normally ambient levels of excitatory amino acids become toxic in the presence of oxidative phosphorylation inhibitors, sodium–potassium pump inhibitors, or potassium-induced partial cell membrane depolarization. Further, excitatory amino acid antagonists such as MK-801 can ameliorate cerebral lesions induced by mitochondrial toxins such as 3-nitropropionic acid (3-NP), 3-acetylpyridine (3-AP), aminooxyactic acid (AOAA), 1-methyl-4-phenylpyridinium (MPP^+), and malo-

nate (Storey *et al.*, 1992; Beal, 1996; Schulz *et al.*, 1996b). The principal indicator of an energetic involvement in the disease process is the observation of insidious weight loss in Huntington's disease patients despite a sustained caloric intake (O'Brien *et al.*, 1990). Subsequently, positron emission tomography (PET) and biochemical studies in postmortem brain have shown selective metabolic defects in brain regions targeted by the disease, and mitochondrial abnormalities in Huntington's disease have been identified in ultrastructural studies of cortical biopsies from juvenile and adult-onset Huntington's disease cases (Goebel *et al.*, 1978).

PET studies show marked reductions in glucose metabolism in the basal ganglia and cerebral cortex of symptomatic Huntington's disease patients (Kuhl *et al.*, 1985; Kuwert *et al.*, 1990; Andrews and Brooks, 1998). Caudate hypometabolism in symptomatic patients has been shown to correlate with clinical test scores for bradykinesia, rigidity, dementia, and functional capacity, while the extent of putaminal hypometabolism correlates with chorea and eye-movement dysfunction, and thalamic hypermetabolism correlates with dystonia scales (Young *et al.*, 1986; Berent *et al.*, 1988; Kuwert *et al.*, 1990). Cortical hypometabolism is also seen in patients suffering psychological disturbances and mood changes, before the onset of motor symptoms (Kuwert *et al.*, 1990). More convincing evidence of a causative role for energetic defects comes from observations of striatal hypometabolism prior to the bulk of tissue loss, and in asymptomatic subjects at risk of developing the disease (Grafton *et al*, 1992; Kuwert *et al.*, 1993; Antonini *et al.*, 1996). Approximately 50% of gene-positive mutation carriers exhibit metabolic defects years before the onset of clinical symptoms (Antonini *et al.*, 1996). PET techniques have also demonstrated that the first clinical symptoms of the disease correlate with loss of 30–40% of striatal dopamine D1 and D2 receptors, which are localized on the GABAergic medium spiny projection neurons in the striatum (Andrews *et al.*, 1997; Hussey *et al.*, 1998). More evidence of metabolic dysfunction in Huntington's disease comes from proton nuclear magnetic resonance (^{1}H NMR) imaging studies which show increased lactate production in the basal ganglia and occipital cortex of Huntington's disease patients (Jenkins *et al.*, 1993, 1998). Notably, these lactate defects can be ameliorated by treatment with the metabolic cofactor coenzyme Q_{10} (Koroshetz *et al.*, 1997).

Biochemical studies in Huntington's disease postmortem tissue have revealed selective dysfunction of components of the oxidative phosphorylation pathway and the tricarboxylic acid (TCA) cycle in brain regions targeted in the disorder. Activities of complexes II-III and IV are markedly reduced in advanced grade Huntington's disease caudate and putamen, while enzyme activities are unaltered in other brain regions (Gu *et al.*, 1996; Browne *et al.*, 1997). Complex I activity is also reported to be impaired in muscle from Huntington's disease patients, but has not been shown to be affected in brain (Parker *et al.*, 1990; Browne *et al.*, 1997; Arenas *et al.*, 1998). Pyruvate dehydrogenase activity is decreased in Huntington's disease basal ganglia and hippocampus, while polarographic studies show that striatal oxygen consumption in Huntington's disease patients is lower than in age-matched controls (Butterworth *et al.*, 1985). The most profound enzyme defect seen in Huntington's disease to date is the dramatic reduction in activity of the TCA cycle enzyme aconitase in affected brain regions and muscle (> 70%; Tabrizi *et al.*, 1999). In addition, mitochondrial toxins, which inhibit succinate dehydrogenase in the TCA cycle and complex II (3-nitropropionic acid and malonate), induce selective striatal lesions in rodents and primates which closely resemble those seen in Huntington's disease (Beal *et al.*, 1993, 1994; Wullner *et al.*, 1994; Schulz *et al.*, 1996a,b). Mitochondrial abnormalities and metabolic defects are also features of other trinucleotide repeat disorders, including SCA1, SCA2, and SCA3, leading to the proposal that energetic dysfunction may play a common role in these disorders, and is directly linked to the polyglutamine defect (Matthew *et al.*, 1993; Mastrogiacomo and Kish, 1994; Mastrogiacomo *et al.*, 1994; Matsuishi *et al.*, 1996).

Further indirect evidence that energetic defects contribute to neurodegenerative processes in Huntington's disease is provided by findings that agents which enhance energy production in the brain exert neuroprotective effects. Preliminary studies in rodent mitochondrial toxin models, and NMR measurements of lactate production in man, suggest that coenzyme Q_{10} and creatine are neuroprotective, putatively via enhancing cerebral energy metabolism (Koroshetz *et al.*, 1997; Matthews *et al.*, 1998). Coenzyme Q_{10} also has potent antioxidant effects. Oral administration of coenzyme Q_{10} improves symptoms in some other mitochondrial-associated disorders including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) and Kearns-Sayre syndrome (reducing cerebrospinal fluid, serum lactate and pyruvate levels, and enhancing mitochondrial enzyme activities in platelets) (Bresolin *et al.*, 1988; Ihara *et al.*, 1989). We recently showed that oral administration of coenzyme Q_{10} ameliorated elevated lactate levels seen in the cortex of Huntington's disease patients, an effect which was reversible on withdrawal of coenzyme Q_{10} (Koroshetz *et al.*, 1997). Furthermore, coenyme Q_{10} attenuates neurotoxicity induced by the mitochondrial toxins MPTP and malonate in animal models (Beal *et al.*, 1994; Schulz *et al.*, 1996b). An alternative strategy is to increase brain energy stores of the high-energy compound phosphocreatine by creatine administration. We recently showed that oral creatine administration in rats attenuates neurotoxicity induced by the succinate dehydrogenase inhibitor 3-NP (Matthews *et al.*, 1998). In addition, increases in cerebral lactate levels and decreases in levels of high-energy phosphate compounds seen in the striata of 3-NP treated rats were attenuated by pretreatment with creatine.

B. Oxidative Damage

Oxidative damage can affect cell viability directly, via oxidation of DNA and other neuronal macromolecules, or indirectly, for example by impairing mitochondrial energy metabolism (Schulz *et al.*, 1996b; Browne *et al.*, 1999a). Evidence for oxidative damage in Huntington's disease is steadily accumulating. Findings include increased incidence of DNA strand breaks, exacerbated lipofuscin accumulation (a marker of lipid peroxidation), elevated DNA oxidative damage products such as 8-hydroxydeoxyguanosine (OH8dG), and increased immunohistochemical staining of oxidative damage products in Huntington's disease striatum and cortex, including staining for 3-nitrotyrosine (a marker for peroxynitrite-

mediated protein nitration), malondialdehyde (marker for oxidative damage to lipids), heme oxygenase (formed during oxidative stress), and OH8dG (Goebel *et al.*, 1978; Tellez-Nagel *et al.*, 1995; Ferrante *et al.*, 1996; Browne *et al.*, 1997, 1999a).

A potential mechanism of mitochondrial dysfunction is via increased generation of free radicals and oxidants. Free radicals including superoxide ($O_2^{\cdot-}$) and hydroxyl radicals ($HO^{\cdot}$) are constantly produced as by-products of aerobic metabolism, but production increases under circumstances of electron transport chain inhibition or molecular defects (Schapira *et al.*, 1992). These agents can induce oxidative damage to cell macromolecules including DNA, proteins, and lipids by a number of different mechanisms, including DNA strand breaks or formation of DNA adducts (e.g., OH8dG), protein carbonylation, or lipid peroxidation. Potential functional consequences include perturbations in DNA transcription and translation, protein synthesis, enzyme activities, and membrane fluidity. Mitochondria are thought to be particularly vulnerable to oxidative injury since most intracellular free radicals are generated by the mitochondrial electron transport chain. Mitochondrial DNA is extremely susceptible due to its localization in the mitochondrial matrix, lack of protective histones, and limited repair mechanisms (Linnane *et al.*, 1989). Thus any increase in free radical production, for example due to impaired activity of a regulatory enzyme such as Cu/Zn SOD or glutathione peroxidase, could reduce the functional capacity of the respiratory transport chain. In addition, the tricarboxylic acid cycle enzyme aconitase, which is severely affected in Huntington's disease, is a prime target for free radical-mediated oxidative damage. The slow, progressive nature of neuronal injury in neurodegenerative disorders may be explained by cycling of free radicals and mitochondrial dysfunction. We have previously found increased levels of oxidative markers in both Huntington's disease and aminotrophic lateral sclerosis. Nuclear DNA OH8dG levels are significantly elevated in Huntington's disease caudate relative to controls (Browne *et al.*, 1997, 1999a). Further evidence supporting a role for oxidative damage in Huntington's disease is that the energetic defects seen in Huntington's disease brain are similar to those induced in cell culture by peroxynitrite, which preferentially inhibits complex II-III and (to a lesser extent) complex IV activity in the electron transport chain (Bolanos *et al.*, 1995).

VI. State of the Art Approaches: Animal Models Provide Insights into Disease Etiology

A. Mitochondrial Toxin Models

A role for mitochondrial energy metabolism dysfunction in the pathogenesis of neuronal degeneration in Huntington's disease is further supported by observations, in both humans and in experimental animals, that the basal ganglia neurons are particularly vulnerable to mitochondrial toxins. These include the complex II inhibitors 3-NP and malonate, AOAA (complex I), potassium cyanide, and sodium azide (complex IV) (Browne and Beal, 1994). Ingestion of 3-NP, an irreversible inhibitor of succinate dehydrogenase (complex II), produces selective basal ganglia lesions and delayed dystonia in humans (Ludolph *et al.*, 1990). Systemic administration of 3-NP to both rats and primates produces age-dependent striatal lesions which are strikingly similar to those seen in Huntington's disease (Brouillet *et al.*, 1993, 1995). In primates, chronic 3-NP administration produces selective striatal lesions which spare NADPH-d neurons, and induce proliferative changes in the dendrites of spiny neurons. Animals also show both spontaneous and apomorphine-inducible movement disorders resembling Huntington's disease (Brouillet *et al.*, 1995). 3-NP basal ganglia lesions in rats are associated with elevated lactate levels, similar to the increased lactate production seen in Huntington's disease patients (Jenkins *et al.*, 1993). 3-NP lesions can be prevented by prior removal of glutamatergic excitatory corticostriatal inputs by decortication, by glutamate release inhibitors, and by glutmate receptor antagonists, suggesting that 3-NP toxicity is mediated by secondary excitotoxic mechanisms (Beal, 1994a; Schulz *et al.*, 1996b).

Intrastriatal injection of either malonate or 3-NP in rats is also associated with increased oxidative damage. We found that the rate of hydroxyl free radical production is elevated in the striatum, as detected by microdialysis (Schulz *et al.*, 1996a). Increased OH^8dG levels in striatum are also detected following systemic 3-NP administration, and elevated 3-nitrotyrosine concentrations are reported after either systemic 3-NP or intrastriatal malonate injection. Further, the finding that 3-NP-induced lesions and concomitant increases in oxidative damage markers were markedly attenuated in mice overexpressing the superoxide free radical scavenger Cu/Zn superoxide dismutase (SOD1) implies that oxidative free radicals contribute to lesion formation (Beal *et al.*, 1995). Furthermore, malonate and 3-NP striatal lesions were attenuated by free radical spin traps and NOS inhibitors. Inhibition of nitric oxide (NO) generation in mice lacking the gene for the neuronal isoform of NOS (nNOS) also resulted in reduced volume of malonate lesions (Schulz *et al.*, 1996b). Hence there is substantial evidence that nitric oxide-mediated oxidative damage is involved in cell death processes following energetic disruption in these models.

B. Transgenic Mouse Models of Huntington's Disease

One of the major drawbacks of relying on human tissue for asessment of neurological disease progression is the inability to adequately map early events in the disease etiology. Substantial evidence of a causative role in the disorder would be provided by evidence of occurrence prior to symptoms and pathology in models of Huntington's disease. Over the past few years, the development of methodology to generate transgenic mouse lines expressing the physiological phenotypes associated with human gene mutations has provided much needed *in vivo* models to circumvent many of these issues. A number of different groups have developed several different transgenic and "knock-in" mouse models of Huntington's disease, which vary in terms of the transgene incorporation technique employed. As a result, mouse phenotypes vary between lines, the features manifested by the animals depending on the nature of the transgene incorporated (i.e., full-length human mutant huntingtin, or a huntingtin *HD* gene fragment incorporating the mutant region in exon 1, or merely an expansion

inserted into the murine *HD* homolog *Hdh*); CAG repeat length; copy number of the mutant gene incorporated; promoter used, and hence cellular specificity of expression; background strains; and expression levels of the mutant gene. The different mouse lines reported to date are discussed below and listed in Table 49.1, which compares the salient features of each of the models.

1. Transgenic Mice Expressing Full-Length Human Mutant Huntingtin

The *HD89* and YAC72 mice discussed below represent two of the most useful transgenic mouse lines developed to date. Their utility is encumbent on the fact that disease-length huntingtin mutations are expressed in the context of the human

TABLE 49.1 Characteristics of Transgenic and Knock-in Mouse Models Expressing the Huntington Disease Mutation

Mutant mouse lines	Background strain	Promoter	Insert	CAG repeat	Symptom onset (weeks)	NII onset (weeks)	Neuronal loss (at end stage)	Life span (weeks)
HD[a]								
HD16 (Wt)	FVB/N	CMV	Full-length	16+/− (A–E)	None	None	None	Normal
HD48			*HD* cDNA,	48 +/− (B,D)	8	12	Stri, CTX >	29–31
HD89			2–22 copies	48 +/− (C)	25	Stri, CTX,	Thal, Hip	29–31
				89 +/− (A–C)	8	Thal, Hip,	(Not 48C)	29–31
				48B +/+	0–8	CBL		21–23
				89A +/+	0–8			21–23
YAC[b]								
YAC18 (Wt)	FVB/N	Constitutive	Full-length	18	None	None	None	Normal
YAC46			*HD* DNA,	46	42	None	None	N/D
YAC72			1–2 copies	72 (2511 line)	26	None	Stri[j]	N/D
Hdh Knock-in[c]								
Hdh^{Q7} (Wt)	C57B16/J	Murine	Full length	7	None	None	None	Normal
Hdh^{Q50}		endogenous	mouse *Hdh*,	48	None	None	None	Normal
Hdh^{Q92}			CAG insert	90	16	60	None	Normal
Hdh^{Q111}				109		16	None	Normal
CAG Knock-in[d]								
CAG 71	C57B16/J	Constitutive	Mouse *Hdh*	71	12	None	None	N/D
CAG 94			−100 bp,	94	8	None	None	N/D
			CAG insert					
Hdh4/6 Knock-in[e]								
Hdh 6/Q72	RF8	Constitutive	Full length	72	12	None	None	N/D
Hdh 4/Q80	JM-1		mouse *Hdh*,	80	12	None	None	N/D
			CAG insert					
N171[f]								
N171-18Q (Wt)	C57B16/C3	Mouse prion	N-terminal	18	None	None	None	Normal
N171-44Q		protein	truncated	44	None	None	None	Normal
N171-82Q			*HD* cDNA,	82	14	21–26	Some; CTX,	21–26
			171 aa				Stri, Hip, CBL	
L63[g]								
L63-46	SJL/B6	Rat NSE	N-terminal	46	12+	N/D	None	N/D
L63-100			fragment,	100	12	CTX, Stri	Stri	N/D
			epitope tag					
R6[h]								
Hdex6 (Wt)	C57B16/CBA	Constitutive	1.9-kb *HD*	18	None	None	None	Normal
Hdex27 (Wt)			DNA	18	None	None	None	Normal
R6/0			fragment	142	None	None	None	Normal
R6/1				113	16–20	Yes	Atrophy/loss?	
R6/2				144	8	3.5	Atrophy/loss?	17–25
R6/5				128–156 +/+	36	Yes	Atrophy/loss?	
				128–156 +/−	None	None	None	Normal
Tet-Off[i]								
		CamKII	Full length		Tet-on	Yes, reversible	Atrophy/loss?	N/D
			mouse *Hdh*,					
			CAG insert					

Note. The models listed reflect those reported by February 2000. CBL, cerebellum; CTX, neocortex; Hip, hippocampus; N/D, not determined to date; NII, neuronal intranuclear inclusions; Stri, striatum; Tet, tetracyclin; Thal, thalamus; wks, weeks of age; Wt, wild type.

[j]Evident in striatal medium spiny neurons by 52 weeks of age.

full-length gene inserted into the mouse genome, rather than just a gene fragment. In contrast to some other putative Huntington's disease models (such as the *Hdh* knock-in mice and R6/2 mice), animals develop region-specific neuronal degeneration over their life span, which provides a suitable context for measuring the efficacy of potential neuroprotective agents.

a. HD48 *and* HD89 *Mice.* Tagle and colleagues generated mice expressing 16 (wild type), 48, and 89 glutamines from full-length human huntingtin cDNA constructs. Using the human promoter, huntingtin expression is widespread throughout the brain and periphery. In these mice, copy number of the gene incorporated varied from two to six in mutant CAG lines. Both 48 and 89 CAG repeat mice showed motor deficits from an early age, developing foot clasping and stereotypic hyperkinetic activity, followed by hypokinesis and locomotor deterioration. Mice die prematurely (24–32 weeks). By about 24 weeks of age marked neuronal cell loss and astrogliosis is evident in the striata of both *HD48* and *HD89* mice, but few neuronal intranuclear inclusions occur. The lack of a distinct correlation between CAG repeat number and disease progression is thought to be associated with the levels of expression in each of the mouse lines, which vary from approximately endogenous levels in 89/89 mice to fivefold higher in 48/48 mice (Reddy *et al.*, 1998).

b. YAC HD *Tg Mice.* Hodgson *et al.* (1999) used yeast artificial chromosome (YAC) technology to generate transgenic mice expressing normal (YAC18: 18 glutamines) and mutant huntingtin (YAC46 and 72: 46 and 72 glutamines, respectively). Mutations are expressed in the context of full-length huntingtin protein. By 12 months of age YAC72 mice have a selective degeneration of medium spiny neurons in the lateral striatum (similar to the selective cell death seen in Huntington's disease patients). Neurodegeneration seems to require nuclear translocation of N-terminal huntingtin fragments, but not neuronal intranuclear inclusion formation. Both YAC46 and YAC72 develop progressive electrophysiological abnormalities at approximately 7 months of age that precede nuclear translocation of huntingtin and cell death. Behavioral changes are manifest in YAC72 mice at 7 months of age.

2. "Knock-in" Mice Expressing Full-Length Huntingtin

"Knock-in" mice arguably represent an excellent model system for investigating the effects of the huntingtin mutation, since the mutation is expressed in the context of the full-length murine huntingtin analog, *Hdh*. Hence, the system uses the endogenous promoter to produce protein at normal murine expression levels, so any differences between animals may be attributed to different polyglutamine repeat lengths. In reality their utility is confounded to some extent by the fact that they do not develop overt, quantifiable, neurodegenerative, or behavioral changes over the animals' life spans. However, as discussed below, there is some evidence of cellular dysfunction associated with the CAG expansion in these models which may be useful as testable parameters for investigating disease mechanisms.

The reason for the lack of neuropathological features of Huntington's disease in these models is thought to result from the combination of low expression levels of the gene and the short life span of mice. In support of this hypothesis, HD48 mice, which do show selective cell death, neuronal intranuclear inclusion, cytoplasmic inclusion formation, and motor disturbances, express up to 5 × wild-type endogenous levels of full-length huntingtin protein (Reddy *et al.*, 1998). However, in the knock-in lines only endogenous murine levels of huntingtin are expressed. Also, the CAG repeat number required to confer toxicity in mice seems to be longer than in humans, perhaps reflecting the short life span of the animals.

a. Hdh *knock-in Mice.* White *et al.* (1997) developed a mouse model of Huntington's disease by extending the polyglutamine tract of the murine homolog (*Hdh*) of the human huntingtin gene (*HD*). CAG repeats from an *HD* chromosome were inserted into the appropriate position in *Hdh* exon 1, altering the mouse *HD* homolog to encode huntingtin protein with 50, 92, or 111 glutamine residues, instead of the 7 normally found in the mouse protein. The transgene has the endogenous promoter, and mice express wild-type levels of huntingtin protein. Mice homozygous for mutant huntingtin do not exhibit any behavioral symptomatology up to 18 months of age. However, recent observations have shown CAG length-dependent translocation of huntingtin protein from cytosol to the nucleus, and eventual neuronal intranuclear inclusion formation in Hdh^{Q92} and Hdh^{Q111} mice (Wheeler *et al.*, 2000). Although there is no evidence of selective cellular pathology in any of these mouse lines yet, we have found that cerebral glucose metabolism and mitochondrial enzyme activities are impaired in Hdh^{Q92} mice at 4 months of age (Browne *et al.*, 1999b, and unpublished observations). This corresponds with a time point preceding neuronal intranuclear inclusion formation in these mice, and may be indicative of early bioenergetic changes associated with the huntingtin mutation (Fig. 49.2, see color insert).

b. CAG71 *and* CAG94 *Mice.* Similar to the technique in *Hdh* mice, the endogenous murine *Hdh* gene was modified by replacing a portion of mouse exon 1 and the adjacent intron with a human sequence containing an expanded CAG repeat region (71 or 94) from a juvenile Huntington's disease lymphoblastoid cell line (Levine *et al.*, 1999). Although no overt cell loss has been reported to date in this model, there is evidence of neuronal dysfunction in CAG94 knock-ins, which showed an increased sensitivity of cortical and striatal cells to *N*-methyl-D-aspartate (NMDA) receptor activation. In contrast, mice expressing fewer glutamines (94) CAG71 knock-ins were less affected than CAG94 knock-ins, showing NMDA responses similar to littermate controls. No cerebral neuronal intranuclear inclusions have been detected in either mouse line.

c. Hdh4/Q80 *and* Hdh6/Q72 *Mice.* Another knock-in Huntington's disease model was generated by Shelbourne and colleagues (1999). They inserted an expanded CAG repeat (72–80) into the murine *Hdh* by homologous recombination and generated two heterozygous knock-in lines, *Hdh4/Q80* and *Hdh6/Q72*. These mice do not develop any neuropathological abnormalities at the cellular level by 17 months of age, but do exhibit a 10–15% reduction in brain size, which is evident by 4 months of age. Neuronal intranuclear inclusions have not been

seen in either line to date, but mice do display an abnormal behavioral phenotype, in the form of chronically increased aggressive behavior toward other mice from about 3 months of age, which is more prevalent in males than in females. There is also evidence of reduced long-term potentiation in hippocampal neurons (Usdin *et al.*, 1999), which the authors suggest reflects the cognitive impairments seen at an early stage in Huntington's disease.

3. Transgenic Mice Expressing Human Mutant Huntingtin Fragments

a. N171 HD Mice. N171 transgenic mice express a truncated portion of Huntington's disease cDNA, consisting of 171 amino acids from the N-terminal, with an expanded glutamine repeat of 44 or 82 (N171-44Q and N171-82Q). Wild-type N171-18Q express 18 glutamines (Schilling *et al.*, 1999). N171-82Q mice display a phenotype of uncoordination, ataxia, and weight loss with onset at approximately 3–4 months, and die prematurely at about 6 months of age. On pathologic examination brains show neuronal intranuclear inclusions when labeled with antibodies to ubiquitin or the N-terminal of huntingtin protein and evidence of neuronal degeneration in the striatum.

b. L63 HD Mice. The L63 mouse line express a FLAG–huntingtin cDNA fusion protein consisting of the first 3221 huntingtin bases from the N-terminal, with 46 or 100 glutamines (Laforet *et al.*, 1998). Motor defects and approximately 20% loss of striatal neuron occur in most mice with 100 CAGs by 6 months of age. Low levels of neuronal intranuclear inclusions are also seen in striatum by 6 months (Laforet *et al.*, 1998; Aronin *et al.*, 1999).

c. R6 HD Mice. The R6 mouse lines were the first Huntington's disease transgenic mice developed and are therefore the best characterized to date. Importantly, it was the development of this model which led to the identification of neuronal intranuclear inclusion formation in the disease paradigm. The R6 mouse lines are transgenic for a fragment of the human *HD* gene, containing 1 kb of the *HD* promoter region and exon 1 containing the abnormal CAG repeat expansion and 262 bp of intron 1 (Mangiarini *et al.*, 1996). The R6/2 line expressing 144 CAGs is the most studied to date, since these animals develop a rapidly progressing neurological phenotype reminiscent of Huntington's disease, before dying prematurely at 17–22 weeks of age. The first motor symptoms occur at about 8 weeks of age, but are preceded by neuronal intranuclear inclusion deposition throughout the brain by 3.5 weeks of age (although it may occur even earlier) (Davies *et al.*, 1997). The motor disorder includes abnormal gait, a resting tremor, abrupt shuddering movements, and stereotypic grooming. However, some features of the disease phenotype do not bear much resemblance to the human disease, including the propensity for seizures in these animals, the lack of overt striatal-specific cell death, and the development of diabetes (Mangiarini *et al.*, 1996; Davies *et al.*, 1997; Hughes *et al.*, 1999; Hurlbert *et al.*, 1999; Sathasivam *et al.*, 1999). Neurotransmitter and receptor abnormalities also occur in R6/2 mice, including dopamine D1 and D2 receptor loss by 8 weeks of age, and alterations in levels of the glutamatergic mGluR1, mGluR2, mGluR3, and mGluR5 metabotropic receptors by 12 weeks of age (Cha *et al.*, 1998). Further, *in situ* studies revealed that D1 and mGluR receptor mRNA is abnormal by as early as 4 weeks of age, preceding the onset of symptoms in these animals. However, contrary to the picture in Huntington's disease brain, GABAergic and NMDA receptor binding levels are not altered in these animals, although AMPA and kainate receptors show some downregulation (Cha *et al.*, 1998). Similarly, neurotransmitter changes do not bear close resemblance to the pattern seen in Huntington's disease. For instance, Reynolds *et al.* (1999) report that striatal GABA levels are unaltered in symptomatic 12-week-old mice, although a slight decrease was observed in the cerebellum. Further, levels of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) are reduced in all brain regions of R6/2 mice, while noradrenaline is decreased in the hippocampus. In contrast, dopamine levels in the striatum are reduced in aged animals, consistent with changes seen in Huntington's disease brain.

As discussed earlier, metabolic defects prior to disease onset are also typical of Huntington's disease. In the R6/2 mouse line, Tabrizi *et al.* (2000) reported that complex II-III and aconitase activities are impaired in the brains of 12-week-old R6/2 Huntington's disease transgenic mouse brains, but these alterations do not precede the onset of the behavioral phenotype and neuronal intranuclear inclusion deposition (S. E. Browne and M. F. Beal, unpublished observations). However, at 12 weeks of age R6/2 mice show an increased vulnerability to metabolic stress, as demonstrated by increased free radical generation and lesion size in response to a 3-NP toxic insult (Bogdanov *et al.*, 1998). Further, we have recently found that treatment of R6/2 mice with creatine, administered in feed from the time of weaning, significantly increases survival and delays brain atrophy, striatal neuron atrophy, and the formation of nuclear inclusions (Ferrante *et al.*, 2000). In addition, prevention of huntingtin cleavage by caspase inhibitors has recently been shown to delay phenotype onset and animal death in this mouse model (Ona *et al.*, 1999).

4. "Inducible" Transgenic Mice Transiently Expressing Mutant Huntingtin

Perhaps the most exciting development in the past few years is the generation of a reversible Huntington's disease mouse model, which incorporates a "tet off" system to modulate expression of the huntingtin gene mutation (Yamamoto *et al.*, 2000). The expression promoter is α-CamKII, which facilitates high levels of expression in the forebrain. Mutant gene expression is under the regulation of a tetracyclin binding sequence, bi-TetO, linked to galactosidase, and then to exon 1 of mutant huntingtin containing an expanded CAG repeat. Tetracyclin binding switches huntingtin transcription off. Yamamoto and colleagues raised mice up to 18 weeks of age in the absence of tetracyclin. Animals developed a severe, progressive motor phenotype, characterized by tremor and foot clasping. Galactosidase staining showed that mutant huntingtin was widely expressed throughout the forebrain in homozygous gene-positive mice, as well as the striatum, hippocampus, hypothalamus, septum, and neocortex. No immunoreactivity was detectable in heterozygote mice. The animals also developed neuronal intra-

nuclear inclusions in all gal-positive regions, but neuronal intranuclear inclusion deposition was limited to brain regions where mutant huntingtin was expressed. Mice went on to develop striatal atrophy, gliosis, and reduced D1 and D2 receptor binding densities in the striatum, indicative of GABAergic cell loss or dysfunction. Most interestingly, all of these parameters of neuronal dysfunction could be reversed, to some extent, by effectively switching expression of the transgene off by treating the animals with the tetracycline analog doxycycline. Animals treated for 4 months showed a marked reduction in the number of neuronal intranuclear inclusions in the striatum and neocortex and partial recovery of striatal atrophy and D1 and D2 binding levels, suggesting that there may be a therapeutic window for disease treatment in postsymptomatic patients. This breakthrough development opens up a plethora of opportunities to observe the consequences of manipulating huntingtin gene expression and to test potential therapeutic strategies.

VII. Conclusions

Although the pathogenic mechanism in Huntington's disease does not seem to be directly linked to the formation of intranuclear huntingtin inclusions, there is an association between the cell death process and CAG repeat length in mutant huntingtin. This is well demonstrated by the observation that incorporation of an expanded CAG repeat stretch (146 CAGs) into a murine gene normally lacking CAG repeats, the hypoxanthine phosphoribosyltransferase gene (*hprt*), resulted in a mouse phenotype reminiscent of other human CAG repeat disorders (JO1 mice, Ordway *et al.*, 1997). Mice develop a progressive neurological phenotype consisting of a late-onset resting tremor, ataxia, decreased open field motor activity, propensity to fall from the rotarod, foot clasping, some incidence of seizures, and premature death at approximately 42–53 weeks of age. CAG length-dependence of these traits was verified by the observations that mice expressing 70 CAG repeats in the *hprt* gene did not develop a behavioral phenotype by 35 weeks of age, whereas the first behavioral symptoms are evident by 12 weeks in JO1 mice (Ordway *et al.*, 1997). A great deal of conjecture now surrounds the question of whether translocation of huntingtin protein into the nucleus, with or without aggregate formation, is an essential step in the pathogenic process in Huntington's disease. Several groups have suggested that huntingtin translocation into the nucleus precedes cell pathology (Saudou *et al.*, 1998; Hackam *et al.*, 1999; Hodgson *et al.*, 1999; Wheeler *et al.*, 1999). Interestingly, in another polyglutamine disease ataxin-1 movement into the nucleus has been shown to be a prerequisite for pathogenesis, and has been associated with gene downregulation in SCA-1 transgenic mice (Lin *et al.*, 2000).

Whereas the definitive pathway underlying cell death in Huntington's disease is yet to be determined, the development and characterization of transgenic and knock-in mouse models of the disorder can only help achieve this goal. In the meantime, it is heartening that initial studies of metabolic enhancers and caspase inhibitors are showing some degree of efficacy in delaying disease onset and extending survival in animal models of the disease.

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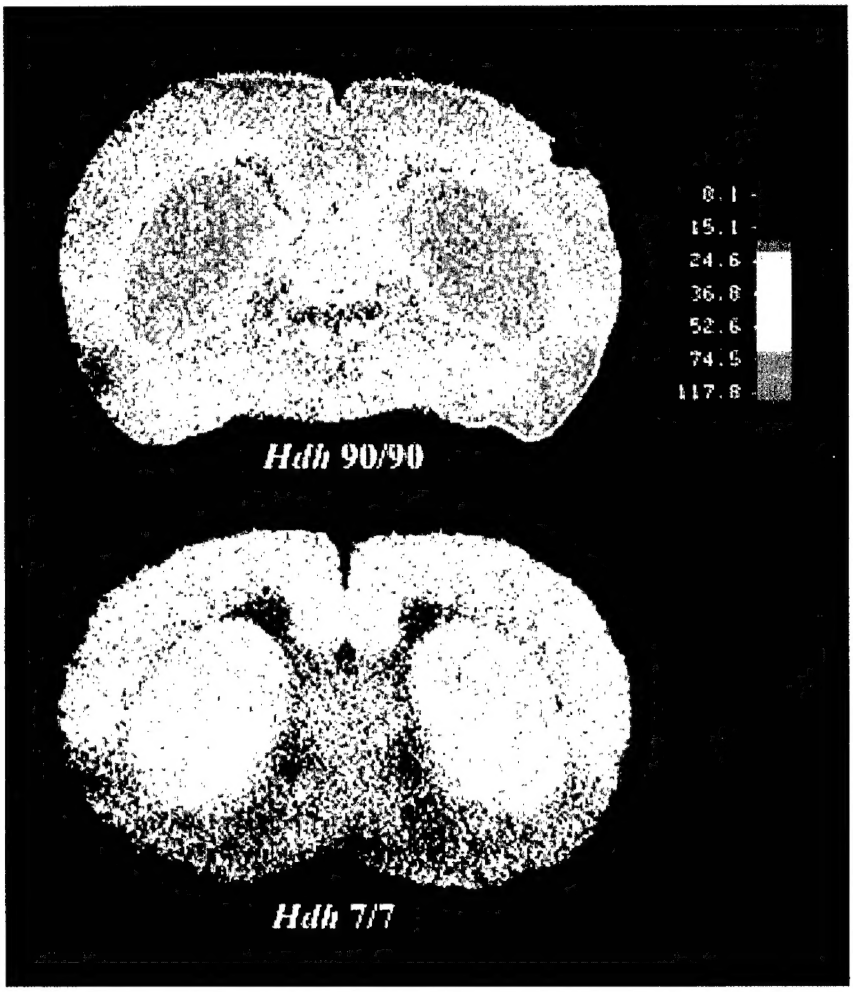

FIG. 49.2 Cerebral glucose use is increased prior to pathological changes in *Hdh* knock-in mice. These representative images are color coded to show local rates of cerebral glucose utilization in the forebrain of *Hdh* knock-in mice at 4 months of age, at the level of the striatum. The color bar illustrates the coding for glucose use (μmol/100 g/min). Glucose use is markedly increased in most forebrain regions of mice expressing 92 glutamines in mutant huntingtin (*Hdh* 90/90, top image) compared to levels in wild-type mice expressing 7 glutamines in huntingtin (*Hdh* 7/7; lower image). These changes in HDH^{Q92} mice precede any behavioral changes, and the formation of neuronal intranuclear inclusions, suggesting that the gene mutation may induce energetic abnormalities at an early stage of the associated disorder (Browne *et al.*, 1999b).

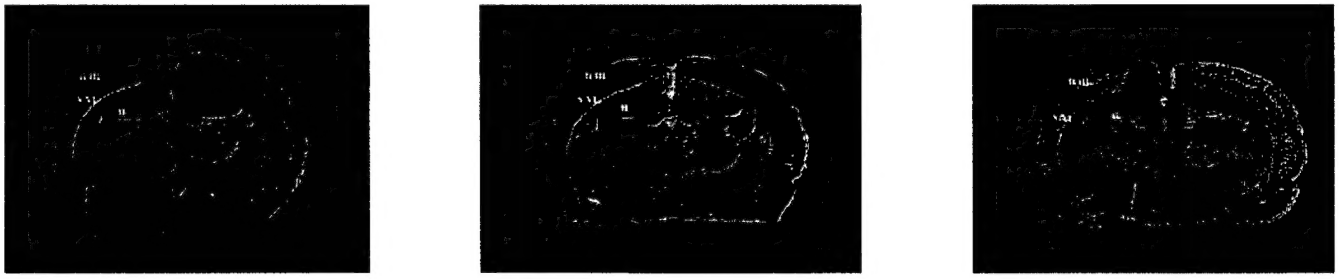

FIG. 63.2 Pseudo-color-enhanced photomicrographs of [^{125}I]IGF-1 binding in hippocampus (H) and layers II/III and V/VI of cortex of 10- (left), 19- (center), and 30-month-old (right) rats. Red indicates the highest level of binding. Data indicate a decline in type 1 IGF receptors in brain with age.